

# PATENT ABSTRACTS OF JAPAN

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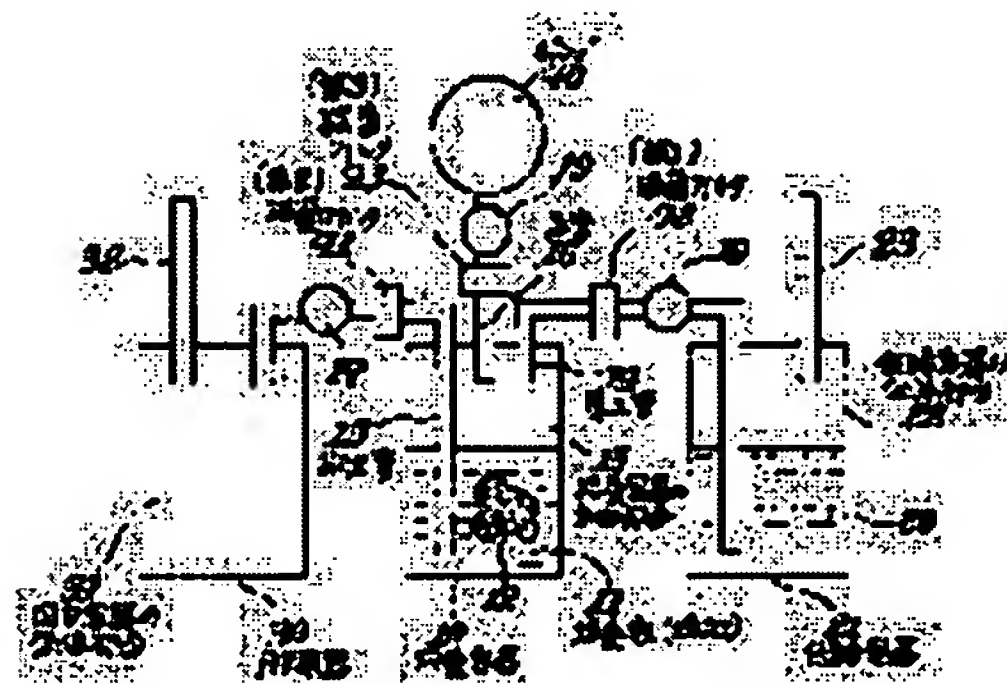
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## (54) CULTURE APPARATUS AND METHOD FOR EXCHANGING CULTURE MEDIUM OF THE SAME



(57)Abstract:

PURPOSE: To provide both a culture apparatus capable of simply exchanging a culture medium while maintaining an aseptic state of a culture container for culturing a cell or a tissue of an organism such as a plant, an animal or a microorganism without damaging the apparatus and a method for exchanging its culture medium.

CONSTITUTION: This culture apparatus is obtained by inserting one end each of an introduction pipe 14 for introducing a new culture medium into a culture container 10, a discharge pipe 13 for discharging the culture medium in the culture container 10 to the outside and a gas pipe 16 making a gaseous part 15 in the culture container 10 communicate with a pump 40 and installing filters 42, 41 and 43 for preventing the entry of microorganisms into the culture container 10 in respective pipes of the introduction pipe 14, discharge pipe 13 and gas

pipe 16 in the culture container 10.

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## CLAIMS

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[Claim(s)]

[Claim 1] In the culture apparatus equipped with the culture container which cultivates the cells and organizations of a living thing, such as vegetation, an animal, and a microorganism Introductory tubing which introduces the liquefied or new culture medium of the letter of a flow into a culture container in said culture container, The end of each tubing of the trachea which opens for free passage the exhaust pipe which discharges the culture medium in a culture container outside, and the gas part and pressure supply means of a culture container is inserted. The culture apparatus characterized by preparing the 1st thru/or the 3rd filter which prevents invasion of the bacillus into a culture container in each duct of said introductory tubing, an exhaust pipe, and a trachea.

[Claim 2] Said culture apparatus is a culture apparatus according to claim 1 which establishes the 2nd pressure supply means which makes the pressure of the gas part of a supply container alternatively higher than the pressure of the gas part of a culture container while having the supply container which holds a still newer culture medium and inserting the other end of said introductory tubing in a supply container.

[Claim 3] Said pressure supply means and said 2nd pressure supply means are a culture apparatus according to claim 2 which is a common gas press pump and establishes the means for switching which connects the gas part of a culture container, or the gas part of a supply container alternatively from this pump.

[Claim 4] Said culture apparatus is a culture apparatus according to claim 1 which establishes the 3rd pressure supply means which makes the pressure of the gas part of the container for recycling alternatively lower than the pressure of the gas part of a culture container while having the container for recycling which collects the culture media in a culture container further and inserting the other end of said exhaust pipe in the container for recycling.

[Claim 5] Said pressure supply means and said 3rd pressure supply means are a culture apparatus according to claim 4 which is a common gas suction pump and establishes the

means for switching which connects the gas part of a culture container, or the gas part of the container for recycling alternatively from this pump.

[Claim 6] Said end of said exhaust pipe is \*\*\*\*(ed) in accordance with the culture container inside periphery, and the end of this exhaust pipe is a removable culture apparatus according to claim 1 to 5 from the body of a culture container in the lid of a culture container.

[Claim 7] The culture-medium exchange approach of the culture apparatus characterized by decompressing the gas part of a culture container with a pressure supply means, and introducing a new culture medium into a culture container through said introductory tubing and said 1st filter after pressurizing the gas part of the culture container of a culture apparatus according to claim 1 with a pressure supply means and discharging the culture medium in a culture container through said exhaust pipe and said 2nd filter.

[Claim 8] The gas part of the culture container of a culture apparatus according to claim 2 or 3 is pressurized with a pressure supply means. After discharging the culture medium in a culture container through said exhaust pipe and said 2nd filter, The culture-medium exchange approach of the culture apparatus which makes the pressure of the gas part of a supply container higher than the gas part of a culture container with the 2nd pressure supply means, and is characterized by introducing a new culture medium into a culture container through said introductory tubing and said 1st filter from a supply container.

[Claim 9] The gas part of the container for recycling of a culture apparatus according to claim 4 or 5 is made lower than the gas part of a culture container with the 3rd pressure supply means. The culture-medium exchange approach of the culture apparatus characterized by decompressing the gas part of a culture container with a pressure supply means, and introducing a new culture medium into a culture container through said introductory tubing and said 1st filter after discharging the culture medium in a culture container in the container for recycling through said exhaust pipe and said 2nd filter.

## DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to liquefied or the method of exchanging the culture medium of the culture apparatus suitable for exchanging the culture medium (culture medium) of the letter of a flow, and a culture apparatus in the culture container which cultivates the cells and organizations of a living thing, such as vegetation, an animal, and a microorganism.

[0002]

[Description of the Prior Art] Conventionally, as a culture container using a solid medium, a petri dish, the bottle, the test tube, the conical beaker, the Erlenmeyer flask, etc. were used, and the lid is put in order to prevent that the mold and saprophytic bacteria in air mix in these culture containers. As a lid, aluminum foil and a heat-resistant bright film are used, some aluminum foil 61 which is a lid as occasionally shown in drawing 6 is used as the membrane filter 62 whose pore size is 0.2-0.45 micrometerphi extent, and there are some which raised the aeration of a culture container, without carrying out saprophytic-bacteria contamination.

[0003] Also when the culture medium of a liquid, i.e., culture medium, is used, it does

not replace as fundamentally as the case of a solid medium, but a Sakaguchi flask may be used so that it can stir well. When cultivating a sample in large quantities especially, a jar fermenter as shown in drawing 7, and in industrial use production, a big cultural tank is used. As the culture-medium exchange approach of the above culture containers, when a solid medium is used, it is carried out by the passage of a culture. That is, a new culture medium is sterilized in a new culture container, and culture media are exchanged by transplanting a culture there. Moreover, when culture medium is used, there is one half omission about culture medium, and there are a tales-doses ON \*\*\*\* approach and the approach of all changing to new culture medium about new culture medium.

[0004]

[Problem(s) to be Solved by the Invention] However, even if it is which approach, in order to maintain an aseptic condition, it is a principle to do all activities in a facility of a clean bench, a clean room, etc., and there is a problem that workability is bad. Moreover, in the case of the commercial jar fermenter, the tube pump is used for installation of culture medium, and discharge, but since traffic and the transportation pressure are low, there is a problem that exchange of culture medium takes time amount. Moreover, in order to convey culture medium, making introductory tubing or an exhaust pipe transform, there is also a problem that degradation of tubing becomes quick. Moreover, when the pump with which culture medium flows directly is used, there is a possibility that a culture-medium component may fix in a pump, and there is also a problem it not only takes the time and effort of cleaning, but that the life of a pump becomes short.

[0005] It was not made in view of this trouble, a clean bench and the clean room of this invention are unnecessary, and it aims at easy and offering the culture apparatus for which a culture medium is exchangeable, and its culture-medium exchange approach, without damaging equipment, with the aseptic condition of a culture container maintained.

[0006]

[Means for Solving the Problem] In order to attain the above-mentioned purpose, in invention according to claim 1 In the culture apparatus equipped with the culture container which cultivates the cells and organizations of a living thing, such as vegetation, an animal, and a microorganism Introductory tubing which introduces the liquefied or new culture medium of the letter of a flow into a culture container in said culture container, The end of each tubing of the trachea which opens for free passage the exhaust pipe which discharges the culture medium in a culture container outside, and the gas part and pressure supply means of a culture container is inserted, and the 1st thru/or the 3rd filter which prevents invasion of the bacillus into a culture container is prepared in each duct of said introductory tubing, an exhaust pipe, and a trachea.

[0007] Moreover, in invention according to claim 2, while having the supply container which holds a still newer culture medium and inserting the other end of said introductory tubing in a supply container, the 2nd pressure supply means which makes the pressure of the gas part of a supply container alternatively higher than the pressure of the gas part of a culture container is established. Furthermore, in invention according to claim 3, in invention according to claim 2, said pressure supply means and said 2nd pressure supply means are common gas press pumps, and establish the means for switching which connects the gas part of a culture container, or the gas part of a supply container alternatively from this pump.



[0008] Moreover, in invention according to claim 4, while having the container for recycling which collects the culture media which became still older and inserting the other end of said exhaust pipe in the container for recycling, the 3rd pressure supply means which makes the pressure of the gas part of the container for recycling alternatively lower than the pressure of the gas part of a culture container is established. Furthermore, in invention according to claim 5, in invention according to claim 4, said pressure supply means and said 3rd pressure supply means are common gas suction pumps, and establish the means for switching which connects the gas part of a culture container, or the gas part of the container for recycling alternatively from this pump.

[0009] In invention according to claim 6, said end of said exhaust pipe is \*\*\*\*(ed) in accordance with a culture container inside periphery, and the end of this exhaust pipe presupposes that it is removable from the body of a culture container with the lid of a culture container. In invention according to claim 7, after pressurizing the gas part of the culture container of a culture apparatus according to claim 1 with a pressure supply means and discharging the culture medium in a culture container through said exhaust pipe and said 2nd filter, the gas part of a culture container is decompressed with a pressure supply means, and a new culture medium is introduced into a culture container through said introductory tubing and said 1st filter.

[0010] In invention according to claim 8, the gas part of the culture container of a culture apparatus according to claim 2 or 3 is pressurized with a pressure supply means. After discharging the culture medium in a culture container through said exhaust pipe and said 2nd filter, the pressure of the gas part of a supply container is made higher than the gas part of a culture container with the 2nd pressure supply means, and a new culture medium is introduced into a culture container through said introductory tubing and said 1st filter from a supply container.

[0011] In invention according to claim 9, the gas part of the container for recycling of a culture apparatus according to claim 4 or 5 is made lower than the gas part of a culture container with the 3rd pressure supply means. After discharging the culture medium in a culture container in the container for recycling through said exhaust pipe and said 2nd filter, the gas part of a culture container is decompressed with a pressure supply means, and a new culture medium is introduced into a culture container through said introductory tubing and said 1st filter.

[0012]

[Function] In invention claim 1 thru/or given in nine, installation of the new culture medium to a culture container is performed through the 1st filter which prevents invasion of a bacillus. Moreover, installation of a culture medium and discharge are performed by controlling the pressure of the gas part of a culture container by the pressure supply means through the 3rd filter which prevents invasion of a bacillus. Moreover, since the 2nd filter is prepared also in the exhaust pipe of a culture medium, penetration of a bacillus is prevented also when the back flow of a culture medium occurs. Therefore, since invasion of the bacillus into a culture container can be prevented, it is not necessary to perform it in a clean bench or a clean room in the case of exchange. Moreover, since the pressure of the gas part of a culture container is controlled, there is no possibility of damaging tubing which a culture medium does not flow and conveys a pressure supply means and a culture medium in a pressure supply means.

[0013] Moreover, in invention according to claim 6, a culture can be held, and the end of

an exhaust pipe is removing an exhaust pipe to the lid of a culture container, and one, and can perform ejection of a culture easily.

[0014]

[Example] Hereafter, the example of this invention is explained using a drawing.

Drawing 1 is the outline block diagram of the culture apparatus concerning the example of this invention. The culture apparatus of this example is equipped with the culture container 10 which cultivates the cells and organizations of a living thing, such as vegetation, an animal, and a microorganism, and culture medium 11 and a culture 12 are contained in the culture container 10. While one edge each of the introductory tubing 14 for introducing culture medium 11 into the culture container 10 and the exhaust pipe 13 which discharges culture medium 11 from the culture container 10 is inserted, respectively, the end of the trachea 16 which opens the gas part 15 and pump 40 of the culture container 10 for free passage is inserted in the culture container 10.

[0015] It is extended in the container 30 for recycling which the other end of the introductory tubing 14 is extended in the new culture medium 20 held into the supply container 21, and collects temporarily the culture medium with which the other end of an exhaust pipe 13 was discharged. In addition, in the supply container 21 and the container 30 for recycling, in order to make the pressure of the gas parts 22 and 31 of each container equal to atmospheric pressure, the tracheae 23 and 32 for making the open air open for free passage are introduced.

[0016] While the valves 18, 19, and 17 which open and close a duct are formed in each duct of the introductory tubing 14, an exhaust pipe 13, and a trachea 16, the sterilization filters 42, 41, and 43 which prevent invasion of the bacillus into the culture container 10 are formed in each duct of the introductory tubing 14, an exhaust pipe 13, and a trachea 16. As sterilization filters 41, 42, and 43, it is good preferably to consider as a filter with a pore size of 0.2-0.45 micrometers. Moreover, any of an automatic valve and a hand valve are sufficient as valves 17, 18, and 19.

[0017] Next, an operation of this example is explained. The parts of the introductory tubing 14, an exhaust pipe 13, and a trachea 16 which are in the culture container 10 side from filters 42, 41, and 43 at least are sterilized by approaches, such as sterilization within an autoclave, dry sterilization, EGO gas, or gamma irradiation, in the culture container 10 list by which filters 42, 41, and 43 and culture medium 11 were included first. And a culture 12 is put in in the culture container 10, and culture begins. It is not necessary to necessarily close valves 17, 18, and 19 at the time of culture. However, if the long duration valve is changed into the open condition, since culture medium 11 will become easy to evaporate, the concentration of culture medium 11 may become high.

[0018] If culture is continued, since it is assimilated with a culture 12, the need of exchanging for new culture medium will come out of various kinds of nutrients contained in culture medium 11. When exchanging, first, valves 17, 18, and 19 are changed into open, close, and an open condition, respectively, from a pump 40, air is sent out to the gas part 15 of the culture container 10, it is pressurized, and the pressure of the gas part 15 is made higher than atmospheric pressure. The culture medium 11 which became old is discharged by this actuation in the container 30 for recycling through an exhaust pipe 13 and a filter 41. At this time, culture medium 11 is discharged by Mr. Fukashi of the culture medium 11 which is inserting the exhaust pipe 13.

[0019] After the old culture medium 11 is discharged, valves 17, 18, and 19 are made

close, open, and open, respectively, the gas of the gas part 15 of the culture container 10 is attracted and decompressed from a pump 40, and the pressure of the gas part 15 is made lower than atmospheric pressure. The new culture medium 20 in the supply container 21 is sent out into the culture container 10 through the introductory tubing 14 and a filter 42 by this actuation.

[0020] In the above operation, since receipts and payments of the culture container 10, culture medium, or air will be altogether performed through the sterilization filters 41, 42, and 43, it can carry out in the usual room, without using a clean bench and a clean room. With a filter 41, also when the back flow of culture medium occurs, penetration of a bacillus is prevented. Moreover, it is not necessary to sterilize the new culture medium 20 and to sterilize a pump 40, the supply container 21, and the container 30 for recycling.

[0021] Drawing 2 shows the 2nd example concerning this invention. The same member as a last example omits the explanation using the same sign. In this example, the gas part 22 of the supply container 21 which holds the new culture medium 20 is connected with the 2nd pump 45 through a trachea 23. Let the 2nd pump 45 be a gas press pump.

[0022] In this example, when carrying out like the 1st example and introducing the new culture medium 20, it operates the 2nd pump 45, and discharge of the old culture medium 11 pressurizes the gas part 22 of the supply container 21, makes the pressure of the gas part 22 higher than the pressure of the gas part 15, and introduces the new culture medium 20 in the supply container 21 into the culture container 10 through the introductory tubing 14 and a filter 42.

[0023] As a modification of this example, it is good also as connecting with the gas part 31 of the container 30 for recycling through a trachea 32 instead of connecting the 2nd pump to the gas part 22 of the supply container 21. Let this 2nd pump be a gas suction pump. And in case the old culture medium 11 is discharged, the 2nd pump is operated, the gas part 31 of the container 30 for recycling is decompressed, the pressure of the gas part 31 is made lower than the pressure of the gas part 15, and the old culture medium 11 is discharged in the container 30 for recycling through an exhaust pipe 13 and a filter 41. Installation of the new culture medium 20 is performed like the 1st example.

[0024] Drawing 3 shows the 3rd example concerning this invention. The same member as a last example omits the explanation using the same sign. In this example, a pump 47 and a directional selecting valve 48 are formed instead of the pumps 40 and 45 of a last example. That is, while connecting with a pump 47 the gas part 22 of the supply container 21 which holds the new culture medium 20 through a trachea 23 and a directional selecting valve 48, the gas part 15 of the culture container 10 is connected with a pump 47 through a trachea 16 and a directional selecting valve 48. Let a pump 47 be a gas press pump.

[0025] When discharging the old culture medium 11, a pump 47 and the gas part 15 are opened for free passage by the directional selecting valve 48, from a pump 47, air is sent out to the gas part 15, it is pressurized, and the culture medium 11 which became old is discharged in the container 30 for recycling through an exhaust pipe 13 and a filter 41. After the old culture medium 11 is discharged, a directional selecting valve 48 is switched, a pump 47 and the gas part 22 are opened for free passage, from a pump 47, air is sent out to the gas part 22, it is pressurized, and the new culture medium 20 is introduced into the culture container 10 through the introductory tubing 14 and a filter 42.

[0026] While connecting a pump to the gas part 15 of the culture container 10 through a



directional selecting valve and a trachea 16 as a modification of this example, it is good also as connecting with the gas part 31 of the container 30 for recycling through a trachea 32. Let a pump be a gas suction pump. In this case, when discharging the old culture medium 11, a pump and the gas part 31 are opened for free passage by the directional selecting valve, air is attracted and decompressed from the gas part 31 with a pump, and the culture medium 11 which became old is discharged in the container 30 for recycling through an exhaust pipe 13 and a filter 41. After the old culture medium 11 is discharged, a directional selecting valve 48 is switched, a pump and the gas part 15 are opened for free passage, air is attracted and decompressed from the gas part 15 with a pump, and the new culture medium 20 is introduced into the culture container 10 through the introductory tubing 14 and a filter 42.

[0027] In addition, in each above-mentioned example, stirring means, such as a screw, are established into the culture container 10, and it may be made to perform stirring of culture medium, and sterilized addition of air. Drawing 4 is the decomposition perspective view showing the concrete structure of the suitable culture container 10 to realize the above-mentioned example and its periphery. In the cylindrical body 10-1 of the culture container 10, the tubular member 13-1 of three merits and demerits, 14-1, and 16-1 are being fixed, the longer one of it constitutes some exhaust pipes 13, and the shorter one constitutes some introductory tubing 14 and a part of trachea 16. O rings 50, 51, 52, and 53 are laid under the opening edge periphery of the cylindrical body 10-1, the tubular member 13-1, 14-1, and 16-1.

[0028] The tubing 13-2 extended to one, 14-2, and 16-2 constitute a part of exhaust pipe 13, introductory tubing 14, and trachea 16 from a lid 10-2, respectively, and the sterilization filters 41, 42, and 43 are formed in the duct. The cylindrical body 10-1 and a lid 10-2 are combined where airtightness is held with O rings 50, 51, 52, and 53 inserted among both. When beginning culture, other supply containers 21 and 30 and pump 40 grade do not need to sterilize that what is necessary is to sterilize only the culture container 10 combined in this way and its periphery. What is necessary is just to connect the free end of tubing 13-2, 14-2, and 16-2 to the remaining exhaust pipes, the introductory remaining tubing, and the remaining trachea after sterilization, respectively.

[0029] Drawing 5 is the decomposition perspective view showing the concrete structure of the suitable culture container 10 to realize the above-mentioned example and its periphery. The culture container 10 consists of a body 10-3 of a culture container, and a lid 10-4, and an exhaust pipe 13-3, the introductory tubing 14-3, and a trachea 16-3 are transfixed to a lid 10-4 by one. The body 10-3 of a culture container and a lid 10-4 are combined by screwing the male screw (10a) formed, respectively and MENEJI (not shown), and airtightness is held with O ring 54 inserted among both.

[0030] The end of an exhaust pipe 13-3 is formed in the shape of TOGURO, and when inserted into the body 10-3 of a culture container, it is \*\*\*\*(ed) along with the inner skin of the body 10-3 of a culture container. Thereby, the function to hold a culture at the end of an exhaust pipe 13-3 can be given. especially -- this maintenance function -- vegetation -- it is suitable in case an adult is cultivated. Moreover, a filter paper and a nonwoven fabric can be placed over the part of the shape of this TOGURO, and it can also consider as culture maintenance material. Since exhaust pipes 13-3 are a lid 10-4 and one, ejection of a culture can be easily performed by removing an exhaust pipe 13-3 with a lid 10-4.



[0031] According to the above example, it has the following effectiveness.

- Culture medium can be exchanged easily, without requiring a special facility of a clean bench, a clean room, etc.
- As compared with the conventional tube pump, it is exchangeable in a short time.
- Since after exchange can use the same object as exchange before, a culture container does not require cost.
- Since culture medium does not flow in a pump, it is not necessary to clean a pump frequently and there is also no possibility that it may be damaged.
- Since it is not necessary to take the lid of a culture container in the case of culture medium exchange, it does not carry out saprophytic-bacteria contamination.

[0032] In addition, in the above example, it is applicable not only like liquid-like culture medium but the culture medium of the letter of a flow (gel) which can be conveyed with a pump.

[0033]

[Effect of the Invention] As explained above, installation of claim 1 thru/or invention \*\*\*\*\* given in nine, and the new culture medium to a culture container It is carried out through the 1st filter which prevents invasion of a bacillus, and installation of a culture medium and discharge are performed by controlling the pressure of the gas part of a culture container by the pressure supply means through the 3rd filter which prevents invasion of a bacillus. Since the 2nd filter is prepared also in the exhaust pipe of a culture medium and penetration of a bacillus is prevented also when the back flow of a culture medium occurs, invasion of the bacillus into a culture container can be prevented and it is not necessary to perform it in a clean bench or a clean room in the case of exchange. Therefore, culture media can be exchanged easily, with the aseptic condition of a culture container maintained.

[0034] Moreover, since the pressure of the gas part of a culture container is controlled, there is no possibility of damaging tubing which a culture medium does not flow and conveys a pressure supply means and a culture medium in a pressure supply means. Moreover, in invention according to claim 6, a culture can be held, and the end of an exhaust pipe is removing with the lid of a culture container, and can perform ejection of a culture easily.

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## DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]

[Drawing 1] It is the outline block diagram of the culture apparatus concerning the example of this invention.

[Drawing 2] It is the outline block diagram of the culture apparatus concerning the 2nd example of this invention.

[Drawing 3] It is the outline block diagram of the culture apparatus concerning the 3rd example of this invention.

[Drawing 4] It is the decomposition perspective view showing the concrete structure of a suitable culture container to realize the example of this invention, and its periphery.

[Drawing 5] It is the decomposition perspective view showing other concrete structures of a suitable culture container to realize the example of this invention, and its periphery.

[Drawing 6] The conventional culture container is shown.

[Drawing 7] The conventional cultural tank is shown.

[Description of Notations]

10 Culture Container

10-4 Lid

11 Culture Medium (Culture Medium)

13 Exhaust Pipe

13-3 End of Exhaust Pipe

14 Introductory Tubing

15 Gas Part of Culture Container

16 Trachea

21 Supply Container

22 Gas Part of Supply Container

30 Container for Recycling

31 Gas Part of Container for Recycling

40, 45, 47 Pump

41, 42, 43 Sterilization filter

48 Directional Selecting Valve

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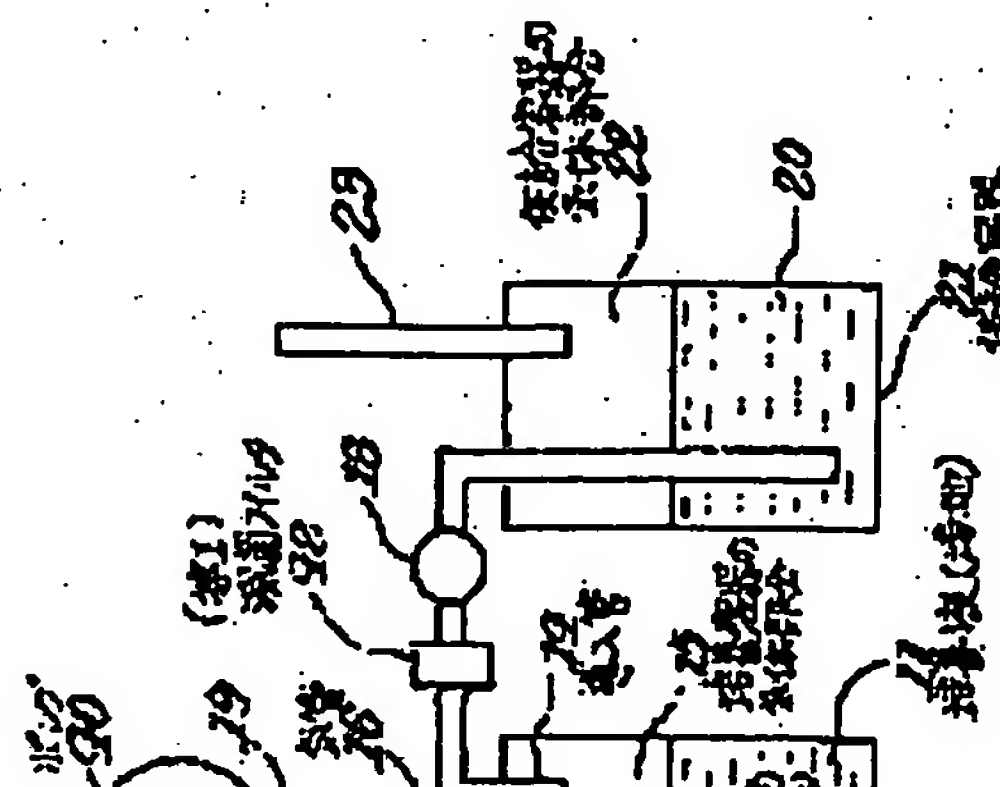
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(54) 【発明の名称】 培養装置及びその培地交換方法

(57) 【要約】

【目的】 植物、動物、微生物等の生物の細胞や組織を培養する培養容器の無菌状態を保ったまま、簡単且つ装置を損傷させることなく培地を交換することができる培養装置及びその培地交換方法を提供する。

【構成】 培養容器10内に、新たな培地を培養容器10に導入する導入管14と、培養容器10の培地を外部に排出する排出管13と、培養容器10の気体部分15とポンプ40とを連通する気管16の各一端を挿入し、前記導入管14 排出管13及び気管16の夫々の管端





(2)

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## 【特許請求の範囲】

【請求項1】 植物、動物、微生物等の生物の細胞や組織を培養する培養容器を備えた培養装置において、前記培養容器内に、液状または流動状の新たな培地を培養容器に導入する導入管と、培養容器内の培地を外部に排出する排出管と、培養容器の気体部分と圧力供給手段とを連通する気管の各々の管の一端を挿入し、前記導入管、排出管及び気管の夫々の管路に培養容器内への菌の侵入を阻止する第1ないし第3フィルタを設けることを特徴とする培養装置。

【請求項2】 前記培養装置は、さらに新たな培地を収容する供給容器を備え、供給容器に前記導入管の他端が挿入されると共に、供給容器の気体部分の圧力を選択的に培養容器の気体部分の圧力よりも高くする第2の圧力供給手段を設ける請求項1記載の培養装置。

【請求項3】 前記圧力供給手段と前記第2の圧力供給手段は、共通の気体圧縮ポンプであり、該ポンプから選択的に培養容器の気体部分または供給容器の気体部分とを接続する切換手段を設ける請求項2記載の培養装置。

【請求項4】 前記培養装置は、さらに培養容器内の培地を回収する回収容器を備え、回収容器に前記排出管の他端が挿入されると共に、回収容器の気体部分の圧力を選択的に培養容器の気体部分の圧力よりも低くする第3の圧力供給手段を設ける請求項1記載の培養装置。

【請求項5】 前記圧力供給手段と前記第3の圧力供給手段は、共通の気体吸引ポンプであり、該ポンプから選択的に培養容器の気体部分または回収容器の気体部分とを接続する切換手段を設ける請求項4記載の培養装置。

【請求項6】 前記排出管の前記一端は、培養容器内面に沿って巻回されており、該排出管の一端は培養容器の蓋と共に培養容器本体から着脱可能である請求項1ないし5のいずれかに記載の培養装置。

【請求項7】 請求項1記載の培養装置の培養容器の気体部分を圧力供給手段によって加圧し、培養容器内の培地を前記排出管及び前記第2フィルタを通して排出した後、培養容器の気体部分を圧力供給手段によって減圧し、新たな培地を前記導入管及び前記第1フィルタを通して培養容器に導入することを特徴とする培養装置の培地交換方法。

【請求項8】 請求項2または3記載の培養装置の培養

管及び前記第2フィルタを通して回収容器に排出した後、培養容器の気体部分を圧力供給手段によって減圧し、新たな培地を前記導入管及び前記第1フィルタを通して培養容器に導入することを特徴とする培養装置の培地交換方法。

## 【発明の詳細な説明】

## 【0001】

【産業上の利用分野】 本発明は、植物、動物、微生物等の生物の細胞や組織を培養する培養容器内の液状または流動状の培地（培養液）を交換するのに適した培養装置、及び培養装置の培地を交換する方法に関する。

## 【0002】

【従来の技術】 従来、固体培地を用いた培養容器としては、シャーレ、瓶、試験管、コニカルビーカー、三角フラスコ等が用いられ、これらの培養容器に空気中のカビや雑菌が混入することを防止するべく、蓋をかぶせている。蓋としては、アルミホイルや耐熱性の透明フィルムが使用され、時には図6に示したように蓋であるアルミホイル61の一部をボアサイズが0.2～0.45μm程度のメンブレンフィルタ62とし、雑菌汚染せずに培養容器の通気を向上させたものもある。

【0003】 液体の培地、即ち、培養液を用いた場合も、固体培地の場合と基本的には代わらないが、その他に良く攪拌できるように坂口フラスコが用いられる場合もある。特に大量に試料を培養する場合には、図7に示したようなジャーファーマンタや、工業用生産の場合には大きな培養タンクが用いられる。以上のような培養容器の培地交換方法としては、固体培地を用いた場合は培養物の継代によって行われる。即ち、新しい培養容器に新しい培地を滅菌しておき、そこに培養物を移植することによって培地の交換を行う。また、培養液を用いた場合は培養液を半分抜き、新しい培養液を同量入れる方法や、全部新しい培養液に入れ換える方法がある。

## 【0004】

【発明が解決しようとする課題】 しかしながら、何れの方法であっても、無菌状態を保つために、クリーンベンチやクリーンルーム等の設備の中ですべての作業を行うことが原則であり、作業性が悪いという問題がある。また、市販のジャーファーマンタの場合、培養液の導入・排出にチューブポンプが用いられているが、給液

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備させることなく培地を交換することができる培養装置及びその培地交換方法を提供することを目的とする。

【0006】

【課題を解決するための手段】上記目的を達成するために、請求項1記載の発明では、植物、動物、微生物等の生物の細胞や組織を培養する培養容器を備えた培養装置において、前記培養容器内に、液状または流動状の新たな培地を培養容器に導入する導入管と、培養容器内の培地を外部に排出する排出管と、培養容器の気体部分と圧力供給手段とを連通する気管の各々の管の一端を挿入し、前記導入管、排出管及び気管の夫々の管路に培養容器内への菌の侵入を阻止する第1ないし第3フィルタを設ける。

【0007】また、請求項2記載の発明では、さらに新たな培地を収容する供給容器を備え、供給容器に前記導入管の他端が挿入されると共に、供給容器の気体部分の圧力を選択的に培養容器の気体部分の圧力よりも高くする第2の圧力供給手段を設ける。さらに、請求項3記載の発明では、請求項2記載の発明において、前記圧力供給手段と前記第2の圧力供給手段は、共通の気体圧縮ポンプであり、該ポンプから選択的に培養容器の気体部分または供給容器の気体部分とを接続する切換手段を設ける。

【0008】また、請求項4記載の発明では、さらに古くなった培地を回収する回収容器を備え、回収容器に前記排出管の他端が挿入されると共に、回収容器の気体部分の圧力を選択的に培養容器の気体部分の圧力よりも低くする第3の圧力供給手段を設ける。さらに、請求項5記載の発明では、請求項4記載の発明において、前記圧力供給手段と前記第3の圧力供給手段は、共通の気体吸引ポンプであり、該ポンプから選択的に培養容器の気体部分または回収容器の気体部分とを接続する切換手段を設ける。

【0009】請求項6記載の発明では、前記排出管の前記一端を、培養容器内面周に沿って誘回し、該排出管の一端は培養容器の蓋と共に培養容器本体から着脱可能とする。請求項7記載の発明では、請求項1記載の培養装置の培養容器の気体部分を圧力供給手段によって加圧し、培養容器内の培地を前記排出管及び前記第2フィルタを通して排出した後、培養容器の気体部分を圧力供給

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5記載の培養装置の回収容器の気体部分を第3の圧力供給手段によって培養容器の気体部分よりも低くし、培養容器内の培地を前記排出管及び前記第2フィルタを通して回収容器に排出した後、培養容器の気体部分を圧力供給手段によって減圧し、新たな培地を前記導入管及び前記第1フィルタを通して培養容器に導入する。

【0012】

【作用】請求項1ないし9記載の発明においては、培養容器への新たな培地の導入は、菌の侵入を阻止する第1フィルタを介して行われる。また、培地の導入、排出は菌の侵入を阻止する第3フィルタを介した圧力供給手段によって培養容器の気体部分の圧力を制御することによって行う。また、培地の排出管にも第2フィルタを設けているため、培地の逆流が起きたときも菌の進入を阻止する。従って、培養容器内への菌の侵入は防げるため、交換の際にクリーンベンチやクリーンルーム内で行う必要がない。また、培養容器の気体部分の圧力を制御するので、圧力供給手段内に培地が流れることがなく、圧力供給手段や培地を輸送する管を損傷するおそれがない。

【0013】また、請求項6記載の発明においては、排出管の一端が培養物を保持することができ、排出管を培養容器の蓋と一体に取り外すことで、簡単に培養物の取り出しを行える。

【0014】

【実施例】以下、図面を用いて本発明の実施例を説明する。図1は、本発明の実施例に係る培養装置の概略ブロック図である。本実施例の培養装置は、植物、動物、微生物等の生物の細胞や組織を培養する培養容器10を備えており、培養容器10の中には培養液11と培養物12が入っている。培養容器10には、培養液11を培養容器10に導入するための導入管14、培養液11を培養容器10から排出する排出管13の各一端がそれぞれ挿入されると共に、培養容器10の気体部分15とポンプ40とを連通する気管16の一端が挿入されている。

【0015】導入管14の他端は、供給容器21の中に収容された新しい培養液20の中に伸びており、排出管13の他端は、排出された培養液を一時的に溜めておく回収容器30の中に伸びている。尚、供給容器21及び回収容器30の中には、各容器の気体部分22、31の圧力を大気圧と等しくするために、外気に連通させるた



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【0017】次に本実施例の作用を説明する。はじめにフィルタ42、41、43及び培養液11が包含された培養容器10並びに導入管14、排出管13及び気管16の少なくともフィルタ42、41、43より培養容器10側にある部分を、オートクレーブ内での滅菌、乾熱滅菌、E.G.Oガスまたは紫外線照射などの方法により滅菌しておく。そして培養容器10内に培養物12を入れて培養が開始する。培養時には、必ずしも弁17、18、19を閉じておく必要はない。但し、長時間弁を開の状態にしておくと、培養液11が蒸発し易くなるので、培養液11の濃度が高くなることがある。

【0018】培養を続けていると、培養液11の中に含まれている各種の栄養分は培養物12によって同化されるため、新しい培養液に交換する必要がでてくる。交換を行うときには、まず、弁17、18、19を夫々閉、閉、開状態にし、ポンプ40から培養容器10の気体部分15に空気を送り出して加圧し、気体部分15の圧力を大気圧より高くする。この操作によって古くなった培養液11は、排出管13及びフィルタ41を通して回収容器30の中に排出される。このとき、培養液11は排出管13の挿入している培養液11の深さまで排出される。

【0019】古い培養液11が排出された後、弁17、18、19を夫々閉、閉、開にし、ポンプ40から培養容器10の気体部分15の気体を吸引して減圧し、気体部分15の圧力を大気圧より低くする。この操作によって供給容器21内の新しい培養液20は、導入管14及びフィルタ42を通して培養容器10の中へ送出される。

【0020】以上の作用において、培養容器10と培養液または空気の出し入れは、すべて滅菌フィルタ41、42、43を介して行うことになるので、クリーンベンチやクリーンルームを用いずに通常の部屋で行うことができる。フィルタ41により、培養液の逆流が起きたときも菌の進入を阻止する。また、新しい培養液20を滅菌する必要もなく、ポンプ40、供給容器21、回収容器30を滅菌する必要もない。

【0021】図2は本発明に係る第2実施例を示す。前実施例と同一の部材は同一の符号を用いて、その説明を省略する。本実施例では、新しい培養液20を収容する

【0023】本実施例の変形例として、第2ポンプを供給容器21の気体部分22に接続する代わりに、回収容器30の気体部分31に気管32を介して接続することとしてもよい。この第2ポンプは、気体吸引ポンプとする。そして、古い培養液11を排出する際に、第2ポンプを作動させて回収容器30の気体部分31を減圧して、気体部分31の圧力を気体部分15の圧力より低くして古い培養液11を排出管13及びフィルタ41を通して回収容器30の中に排出する。新しい培養液20の導入は、第1実施例と同様に行う。

【0024】図3は本発明に係る第3実施例を示す。前実施例と同一の部材は同一の符号を用いて、その説明を省略する。本実施例では、前実施例のポンプ40、45の代わりにポンプ47と方向切換弁48を設けたものである。即ち、新しい培養液20を収容する供給容器21の気体部分22を気管23及び方向切換弁48を介してポンプ47と接続すると共に、培養容器10の気体部分15を気管16及び方向切換弁48を介してポンプ47と接続する。ポンプ47は、気体圧縮ポンプとする。

【0025】古い培養液11を排出するときには、方向切換弁48でポンプ47と気体部分15とを連通し、ポンプ47から気体部分15に空気を送り出して加圧し、古くなった培養液11を、排出管13及びフィルタ41を通して回収容器30の中に排出する。古い培養液11が排出された後、方向切換弁48を切り換えて、ポンプ47と気体部分22とを連通し、ポンプ47から気体部分22に空気を送り出して加圧し、新しい培養液20を導入管14及びフィルタ42を通して培養容器10の中へ導入する。

【0026】本実施例の変形例として、ポンプを方向切換弁及び気管16を介して培養容器10の気体部分15に接続すると共に、気管32を介して回収容器30の気体部分31に接続することとしてもよい。ポンプは気体吸引ポンプとする。この場合、古い培養液11を排出するときには、方向切換弁でポンプと気体部分31とを連通し、ポンプで気体部分31から空気を吸引して減圧し、古くなった培養液11を排出管13及びフィルタ41を通して回収容器30の中に排出する。古い培養液11が排出された後、方向切換弁48を切り換えて、ポンプと気体部分15とを連通し、ポンプで気体部分15か



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その長い方が排出管13の一部を、短い方が導入管14の一部及び気管16の一部を構成する。円柱形本体10-1、管状部材13-1、14-1、16-1の開口端周部にはOリング50、51、52、53が埋設される。

【0028】蓋10-2から一体に伸びる管13-2、14-2及び16-2が、夫々排出管13、導入管14及び気管16の一部を構成し、その管路内には滅菌フィルタ41、42及び43が設けられる。円柱形本体10-1と蓋10-2は、両者の間に介挿されるOリング50、51、52、53によって気密性が保持された状態で結合される。培養を始めるときには、このように結合された培養容器10及びその周辺部のみを滅菌すればよく、他の供給容器21、30、ポンプ40等は滅菌を行う必要がない。滅菌後に管13-2、14-2、16-2の遊端を夫々残りの排出管、残りの導入管、残りの気管に接続すればよい。

【0029】図5は、上記実施例を実現するのに好適な培養容器10及びその周辺部の具体的構造を示す分解斜視図である。培養容器10は、培養容器本体10-3と蓋10-4からなり、蓋10-4には一体に排出管13-3、導入管14-3、気管16-3が貫通固定される。培養容器本体10-3と蓋10-4は、夫々形成されたオネジ(10a)とメネジ(図示せず)を螺合することで結合され、両者の間に介挿されるOリング54によって気密性が保持される。

【0030】排出管13-3の一端はトグロ状に形成され、培養容器本体10-3内に挿入されたときに培養容器本体10-3の内周面に沿って巻回される。これにより、排出管13-3の一端に、培養物を保持する機能を持たせることができる。特にこの保持機能は、植物成体を培養する際に適している。またこのトグロ状の部分にわたって濾紙や不織布を置いて培養物保持材とすることもできる。排出管13-3は、蓋10-4と一体であるため、蓋10-4と共に排出管13-3を取り外すことで、簡単に培養物の取り出しを行うことができる。

【0031】以上の実施例によれば、以下の効果を有する。

・クリーンベンチやクリーンルーム等の特別な設備を要することなく、培養液の交換を簡単に行うことができ

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液に限らず、ポンプによって輸送することができる流動状(ゲル状)の培地にも同様に適用することができる。

【0033】

【発明の効果】以上説明したように、請求項1ないし9記載の発明によれば、培養容器への新たな培地の導入は、菌の侵入を阻止する第1フィルタを介して行われ、培地の導入、排出は菌の侵入を阻止する第3フィルタを介した圧力供給手段によって培養容器の気体部分の圧力を制御することによって行われ、培地の排出管にも第2フィルタを設けているため、培地の逆流が起きたときも菌の進入を阻止するので、培養容器内への菌の侵入は防げ、交換の際にクリーンベンチやクリーンルーム内で行う必要がない。従って、培養容器の無菌状態を保ったまま、簡単に培地を交換することができる。

【0034】また、培養容器の気体部分の圧力を制御するので、圧力供給手段内に培地が流れることがなく、圧力供給手段や培地を輸送する管を損傷するおそれがない。また、請求項6記載の発明においては、排出管の一端が培養物を保持することができ、培養容器の蓋と共に取り外すことで、簡単に培養物の取り出しを行える。

【図面の簡単な説明】

【図1】本発明の実施例に係る培養装置の概略ブロック図である。

【図2】本発明の第2実施例に係る培養装置の概略ブロック図である。

【図3】本発明の第3実施例に係る培養装置の概略ブロック図である。

【図4】本発明の実施例を実現するのに好適な培養容器及びその周辺部の具体的構造を示す分解斜視図である。

【図5】本発明の実施例を実現するのに好適な培養容器及びその周辺部の他の具体的構造を示す分解斜視図である。

【図6】従来の培養容器を示す。

【図7】従来の培養タンクを示す。

【符号の説明】

10 培養容器

10-4 蓋

11 培養液(培地)

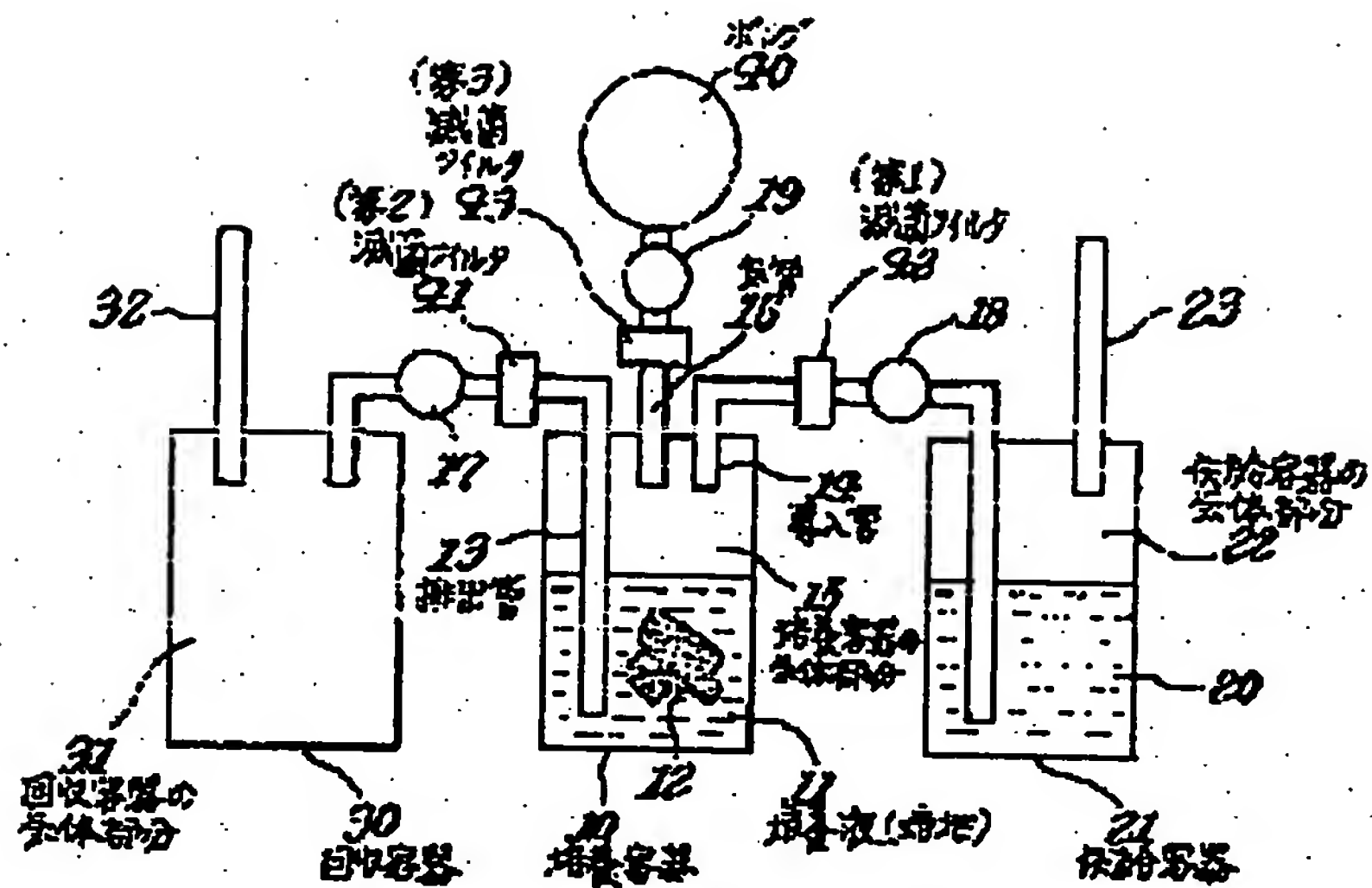
13 排出管

13-3 排出管の一端

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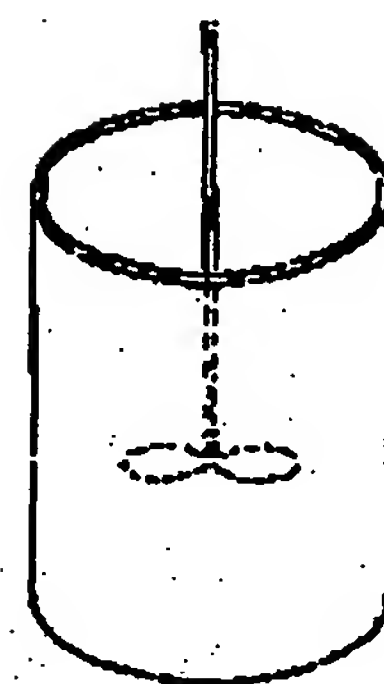
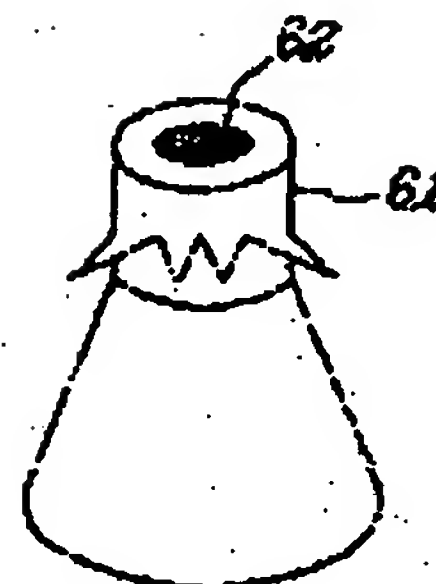
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【図1】

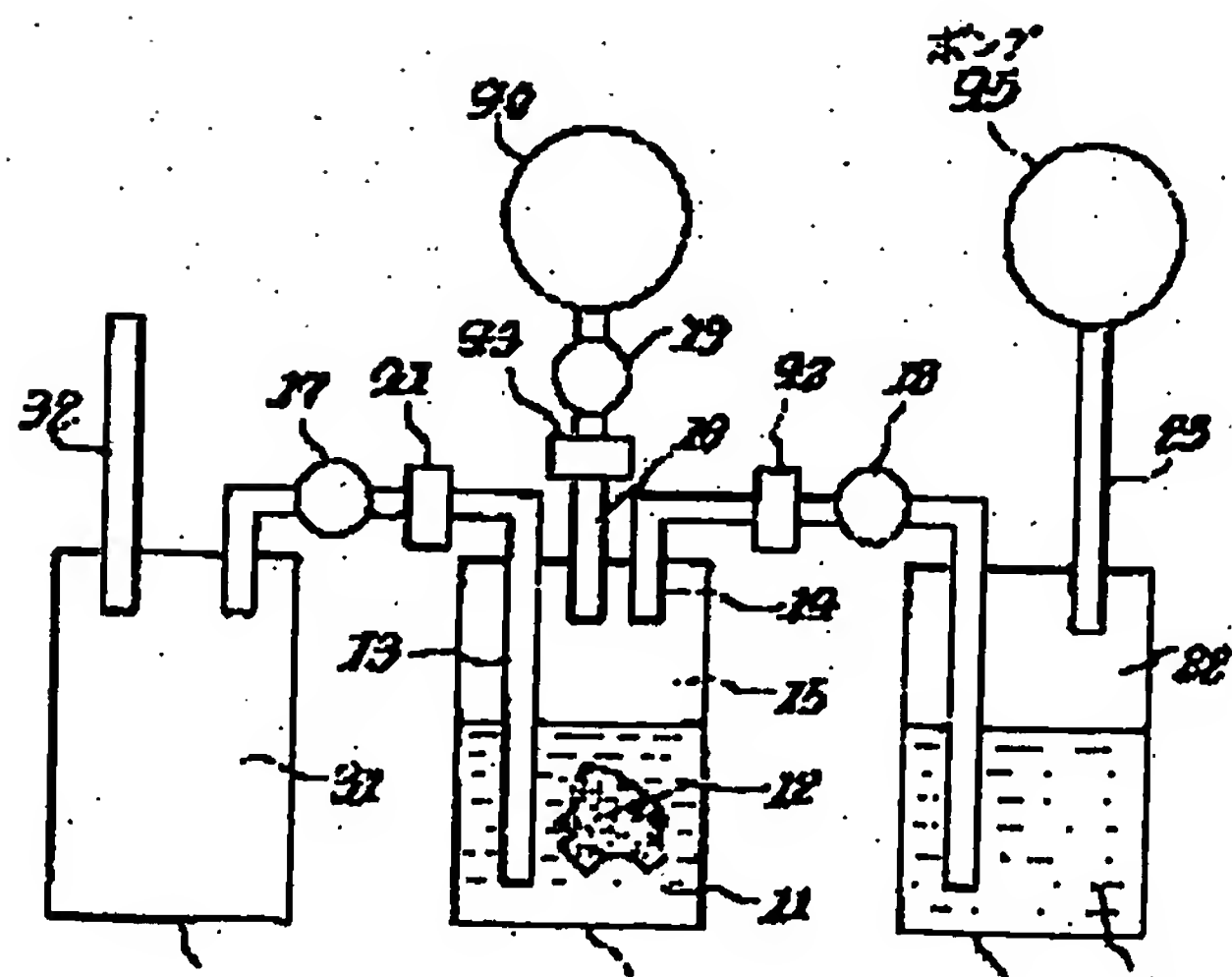


【図6】

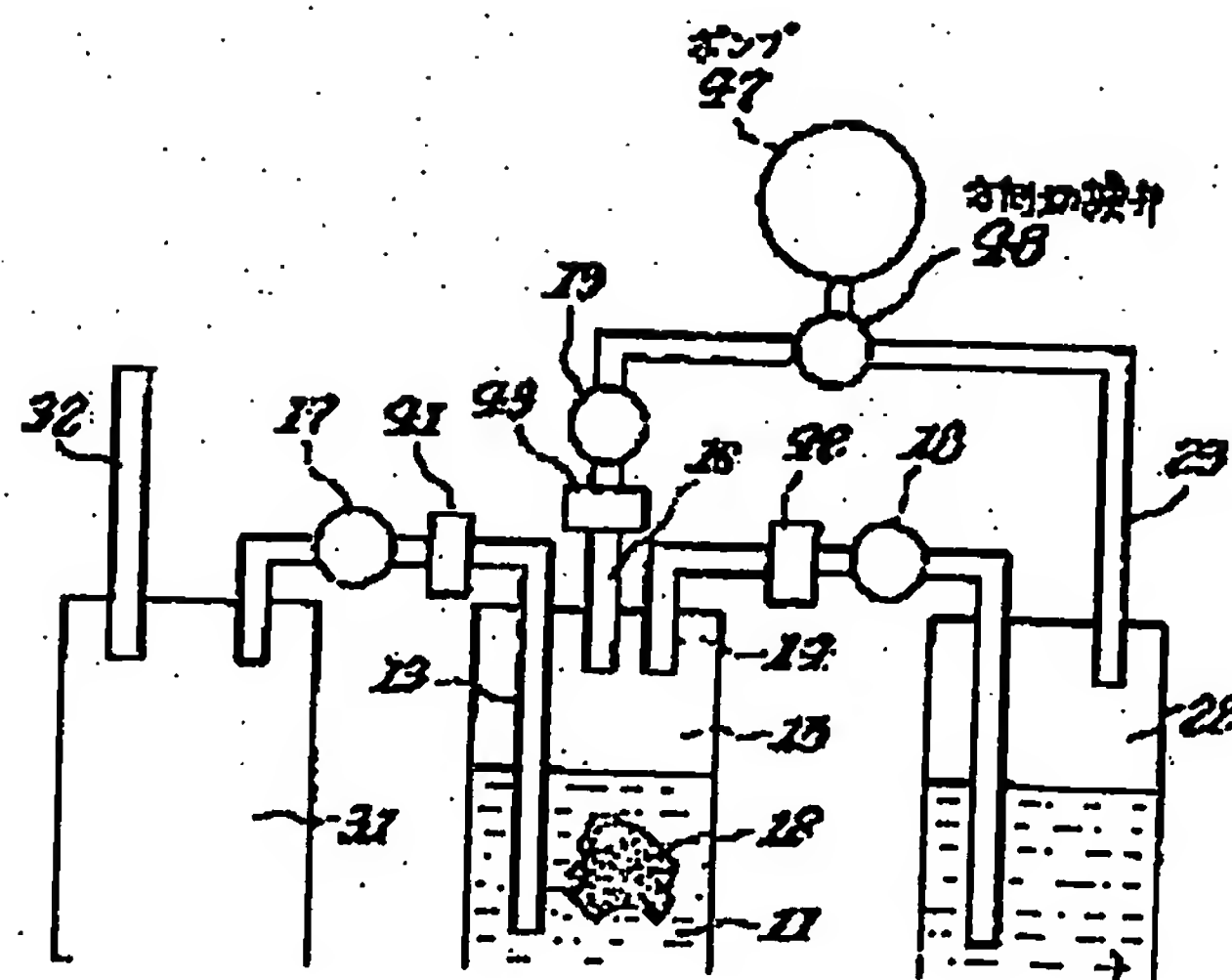
【図7】



【図2】



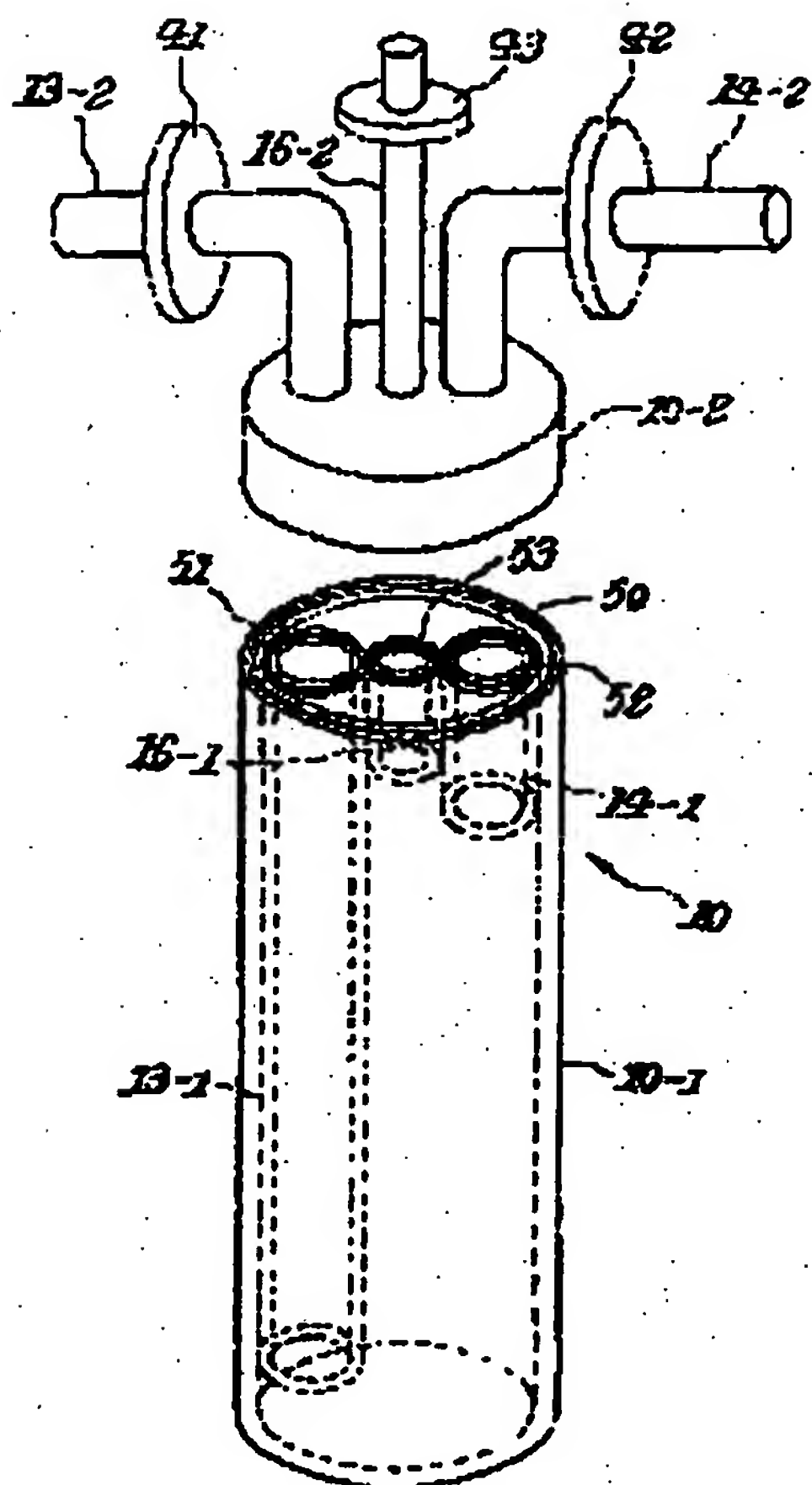
【図3】



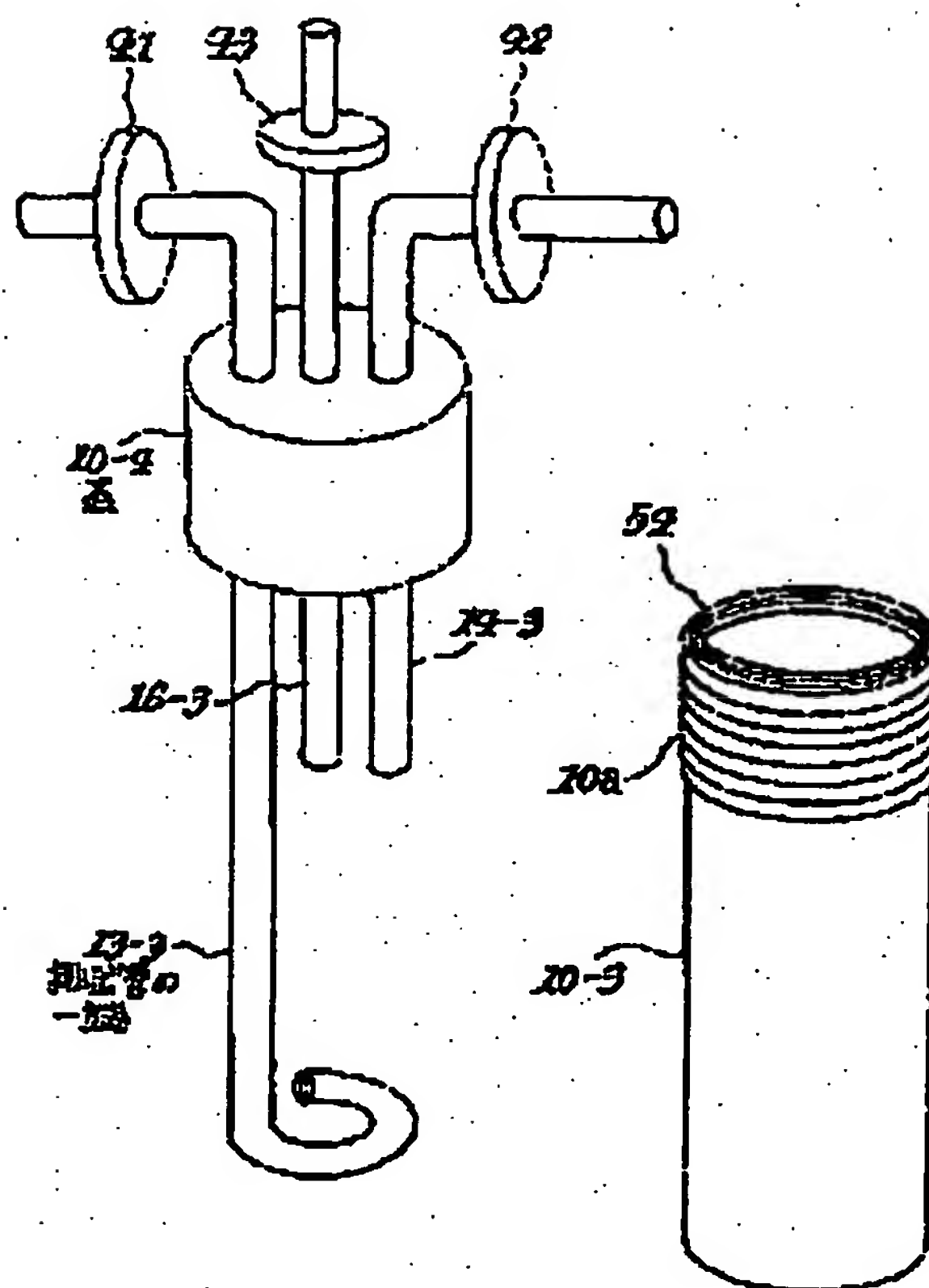
(7)

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【図4】



【図5】



フロントページの続き

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